Microbiome in Multiple Sclerosis: Role of B-cells and Microglia

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INTRODUCTION

Microbiome: is referred to the population of bacteria residing in the gut. Microbiome from MS patients is different than healthy individuals and experimental studies show improvement of EAE in germ-free conditions and upon treatment with oral but not intravenous antibiotics and worsening upon transplantation of feces from MS patients. The cross talk of microbiome and CNS is called gut brain axis (**Fig-1**).

B-cells: play a significant role in MS pathophysiology and this is supported by the significant efficacy of anti-CD20 therapies, presence of oligoclonal bands, presence of B-cells in the demyelinating lesions and formation of meningeal follicles.

Microglia: are the CNS-specific innate immune cells which are also implicated in MS through evidence from genetics, post-mortem histopathological studies as well as advanced positron emission tomography (PET).

Microbiome is essential for proper development and maturation of both microglia and B-cells. The objectives of this study is investigate the association of MS microbiome with B-cells and Microglia.

DISCUSSION

B-cell depleting therapies normalize several bacterial species that are altered in MS, which implies an association between B-cells and microbiome. Microbiota may impact CNS disease by shaping the microglia phenotype.

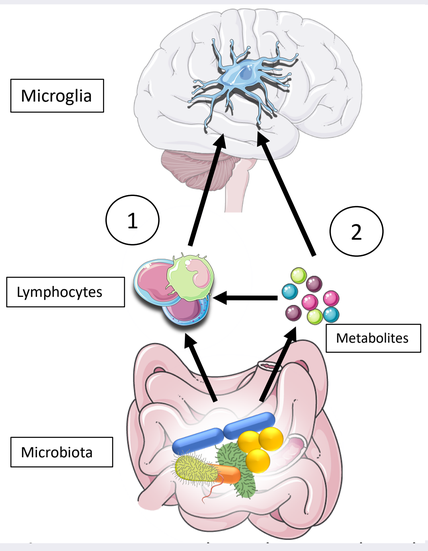

Figure 1- Gut-brain axis.

METHODS

B-cells: We used stool samples from patients treated with anti-CD20 therapies (n=16) and compared them with untreated patients (n=34) and healthy controls (n=49). Patients were recruited as part of the Comprehensive Longitudinal Investigation of MS at Brigham and Women's Hospital (CLIMB). The CLIMB database is an IRB approved study and participants give informed consent to participate.

- Bacterial DNA was extracted from samples using DNeasy PowerLyzer PowerSoil Kit (Qiagen) following manufacturer's instructions.
- The V4 region of the 16S rRNA gene was amplified by polymerase chain reaction and sequenced on the MiSeq platform (Illumina).
- We used Quantitative insights for microbial ecology 2 (QIIME2) to de-multiplex and quality filter sequences, assign taxonomy, calculate alpha-diversity metrics, beta-diversity between samples, calculate relative abundance, and visualize the data.

Microglia: Three sets of untreated relapsing and progressive MS and healthy control with similar age, sex and ethnicity were selected from the CLIMB database. IACUC approval was obtained for all the procedures in the study.

- We used 8-week-old C57B/6 mice and treat them with 3 days of broad-spectrum oral antibiotics [metronidazole (1g/L) + ampicillin (1g/L)+ neomycin(1g/L)+ vancomycin(0.5g/L)] in the drinking water to deplete the microbiota.
- One gram of fecal material from each donor was suspended in 15 mL of pre-reduced anaerobically sterilized saline and frozen in 1 mL aliquots.
- We then orally gavaged 200ul of fecal solution 3x/week for 2 weeks into five mice each (**Fig-2**).
- Microglia were FACS sorted using microglia specific mono-clonal antibody (Fcrls-APC, which is expressed on microglia, but not on infiltrating myeloid cells) as CD11b+, Ly6c-, and Fcrls+.
- Samples were processed with Smart-Seq2 protocol and sequenced on Illumina sequencers. Data were analyzed using DESeq2 for differential expression analysis. A false discovery rate (FDR) cutoff of 5% was used.

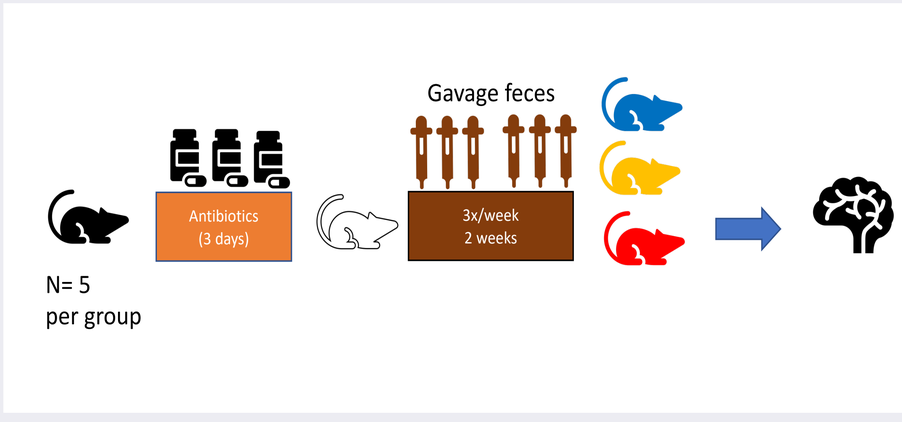

Figure 2- Microglia study design.

RESULTS

B-cells: We found that anti-CD20 treatment normalized multiple species altered in untreated MS patients back to healthy control levels, including increasing *Blautia*, *Roseburia* and *Paraprevotellaceae*, and depleting *Eubacterium dolichum* and *Streptococcus anginosus*. **(Fig-3)**

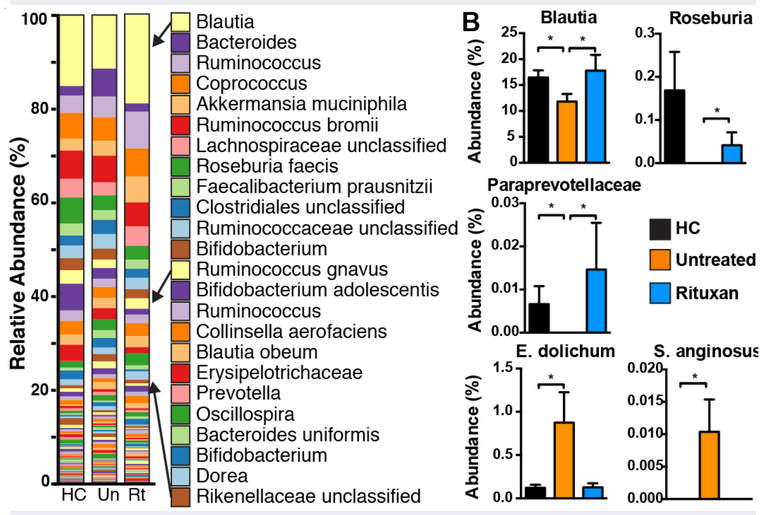

Figure 3- Effects of B-cell depletion on gut microbiota.

Microglia: Fecal microbiome transfer from MS subjects differentially affected the transcriptional profile of microglia in naïve mice compared to healthy controls. 16 genes were upregulated and 20 genes down-regulated following fecal transfer. The down-regulated genes consisted primarily of the genes in the IL-10 pathway. We also observed differences in the gene profile of microglial between fecal transfer from relapsing vs. progressive subjects.

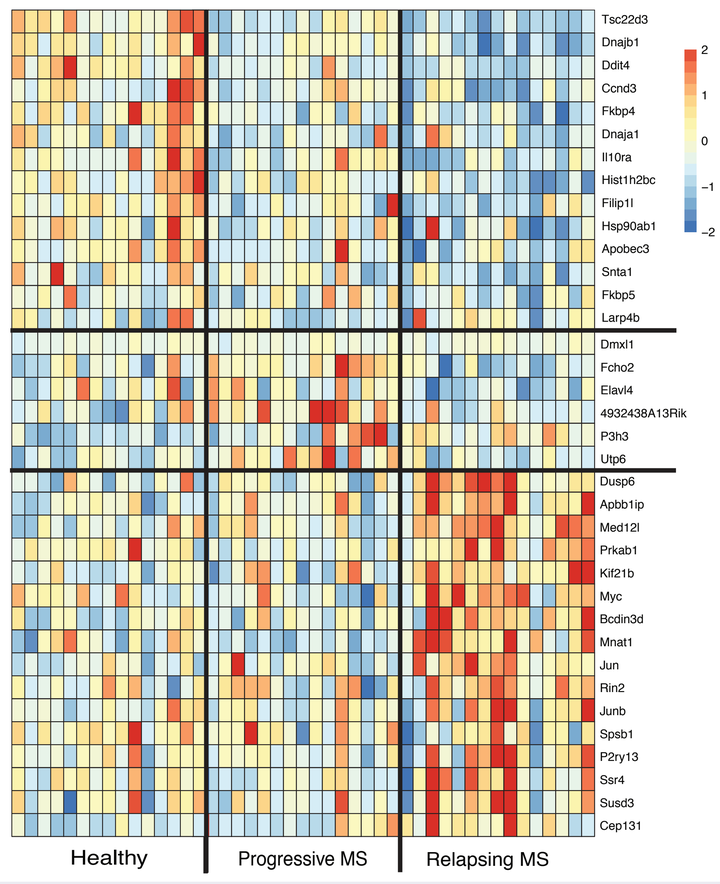

Figure 4- Effects of MS microbiota on microglia gene expression profile.

Literature

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